Phosphorylation reaction+ Blunt ligation

PCR products have blunt ends. For ligation to occur, at least one of the DNA ends (insert or vector) should contain a 5´ phosphate. T4 Polynucleotide Kinase enzyme catalyzes the transfer and exchange of Pi from the γ position of ATP to the 5´ -hydroxyl terminus of polynucleotides.

Phosphorylation reaction:

T4 DNA ligase buffer x10	2.5ul
T4 kinase (PNK)	1ul
DNA (100ng)	X ul
PEG 4000 50%	2.5ul (5%)
DDW	19- X ul

25ul (total vol.)

- Incubate at 37^0C for 30 min.
- **Heat Inactivation prior to ligation**- 65°C for 20 minutes.
- Cool on ice.

Ligation reaction:

- Add to phosphorylation reaction (after cooling) 1ul T4 DNA ligase.
- Incubate 2hr at R.T or 16°C O/N.

Ligation is ready for transformation (heat-shock only).

Read more:

PNK - https://www.neb.com/products/m0201-t4-polynucleotide-kinase